Nordic Council of Ministers

Nordic Working Paper

Alignment and overall strategy for the handling of in silico methods in human health assessment of plant protection products in the Northern Zone

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1. Abbreviations

AE	Assessment element
CLP	Classification, Labelling and Packaging
ECHA	European Chemicals Agency
EFSA	European Food Safety Authority
NAM	New approach methodologies
NGRA	Next generation risk assessment
NKE	Nordic Working Group for Chemicals, Environment, and Health
NZ	Northern Zone
PPP	Plant Protection Product
QAF	(Q)SAR Assessment Framework
QMRF	(Q)SAR model reporting format
QPRF	(Q)SAR prediction reporting format
QRRF	(Q)SAR result reporting format
(Q)SAR	(Quantitative) Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
WoE	Weight of the evidences

2. Preface

In 2022 NKE supported a workshop on alternative *in vitro* methods to vertebrate studies (i.e. *in vivo*) under CLP, held by the Pesticide Northern Zone toxicology group in Helsinki[1]. In line with *in vitro* methods, *in silico* methods, such as (Q)SAR and read-across, are used as alternative approaches to animal testing under the term NAM. The focus of this present NKE supported workshop, was on *in silico* methods, and the potential use of these in the assessment of health effects of pesticides.

For the authorisation and classification of PPPs, Regulation (EC) No. 1107/2009 (on PPPs), in accordance with Regulation (EU) No. 284/2013 (on data requirements for PPPs) and Regulation (EC) No. 1272/2008 (CLP), needs to be considered. According to Regulation (EU) No. 284/2013, use of validated alternative *in vitro* or *in silico* methods should be considered before tests are performed on vertebrate animals. Under Regulation (EC) No. 1272/2008, (Q)SAR and read-across are mentioned as a part of the WoE approach for determination of hazard.

In silico tools, such as (Q)SAR and read-across, are currently integrated in the PPP assessment to a very limited extent, e.g. assessment of genotoxicity of groundwater metabolites. However, these tools may potentially be used in a broader context in the future.

Together with other NAMs, *in silico* methods are a part of NGRA[2]. Currently, activities are on-going with the ultimate goal of phasing out animal studies when feasible[3]. NAMs, including *in silico* methods, are essential in that context and form the basis for NGRA. Use of (Q)SAR and read-across has already been implemented in a defined approach for assessing skin sensitisation, not as a stand-alone, but in combination with other sources of information as a valid method of addressing skin sensitisation[4].

Assessing pitfalls and weighing the importance and quality of the *in silico* predictions may be challenging for regulatory risk assessors. A newly published assessment framework by OECD[5] aims to make the assessment of these predictions more accessible for the regulatory risk assessor, also within the regulatory framework.

The target audience of this workshop was regulatory risk assessors in the NZ working with REACH-, PPP- and CLP regulations. The purpose of this workshop was to make the participants familiar with the use of QAF and its reporting formats, and for participants to be updated on state of the art regarding use of *in silico* tools for assessing PPPs.

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[1] Tägt, J & Ali, I (2023). Workshop on alternative *in vitro* methods to vertebrate studies under CLP. *Nordic working paper, 2023:901*. Retrived from http://dx.doi.org/10.6027/NA2023-901

[2] U.S. EPA (2014). Next Generation Risk Assessment: Incorporation Of Recent Advances In Molecular, Computational, And Systems Biology (Final Report). U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-14/004. Retrieved from: https://cfpub.epa.gov/ncea/risk/recordisplay.cfm?deid=286690

[3] Partnership for the Assessment of Risks from Chemicals (2023). Deliverable D2.3, PARCroute roadmap1, WP 2 – T2.2. P-A-R-C HORIZON-HLTH-2021-ENVHLTH-03 CONTRACT N. 101057014. Retreived from https://www.eu-parc.eu/sites/default/files/2023-10/PARC_D2.3.pdf.

[4] OECD (2023). Guideline No. 497: Defined Approaches on Skin Sensitisation, OECD Guidelines for the Testing of Chemicals, Section 4, *OECD Publishing*, Paris. https://doi.org/10.1787/b92879a4-en.

[5] OECD (2024). (Q)SAR Assessment Framework: Guidance for the regulatory assessment of (Quantitative) Structure Activity Relationship models and predictions - Second edition, OECD Series on Testing and Assessment No. 405, *OECD Publishing*, Paris.

3. Partners and collaborators

The steering group of the workshop constituted Kirstine Lindhardt Sæderup (The Danish Environmental Protection Agency) and Jonas Tägt (Swedish Chemicals Agency). Jörgen Henriksson (Swedish Chemicals Agency) made significant contributions to the development of the case example presented on day 2.
All additional members of the NZ tox group Diana Lilienblatt, Priit Kersen (Agriculture and Food Board, Republic of Estonia), Dalia Janušauskaitė, Audra Paltanavičienė (State Plant Service under the Ministry of Agriculture, Lithuania), Laura Voitina, Una Conka-Priedeslaipa (State Plant Protection Service of the Republic of Latvia), Annike Irene Totlandsdal, Maren Kolltveit Bakkebø, Irene Beate Sørvik Malme, Tirill Medin (Norwegian Food Safety Authority), Marika Päällysaho, Teija Virtamo-Tiiska (Finnish Safety and Chemicals Agency), Madeleine Sjöberg (Swedish Chemicals Agency), Louise Lundberg, Pia Mouritzen Hunter, Kirstine Lindhardt Sæderup (Danish Environmental Protection Agency) provided valuable input to the scope of the workshop and program in the planning process.

The workshop was funded by the NKE.

The workshop was organised on 28-29th October 2024 face-to-face in Odense, Denmark, and with online participants. Kirstine Lindhardt Sæderup, Pia Mouritzen Hunter and Louise Lundberg (The Danish Environmental Protection Agency) were organisers of the workshop.

4. Participants from the Pesticide Northern Zone toxicology group

LT	Dalia Janušauskaitė
LT	Audra Paltanavičienė,
EE	Diana Lilienblatt
EE	Priit Kersen
NO	Tirill Medin
NO	Irene Beate Sørvik Malme
NO	Maren Kolltveit Bakkebøe
NO	Annike Irene Totlandsdal
SE	Jonas Tägt
SE	Madeleine Sjöberg
FI	Marika Päällysaho-Juujärvi
FI	Teija Virtamo-Tiiska
LV	Zane Indzere
LV	Emils Zarins
DK	Kirstine Lindhardt Sæderup
DK	Pia Mouritzen Hunter
DK	Louise Lundberg

5. Workshop summary and outcome

5.1. Background

In general, the NZ member states experience challenges when (Q)SAR and read-across approaches are used for evaluation of e.g. groundwater metabolites both in terms of assessing the applicability of these *in silico* methods and in terms of evaluating the quality of provided (Q)SAR and read-across prediction. Currently, *in silico* methods are often used by applicants to address potential effects of pesticide groundwater metabolites. In the future, *in silico* methods will most likely be used more frequently for addressing data gaps in the WoE approach when assessing PPPs, as alternatives to vertebrate studies. It is crucial for human health risk assessors in the Northern Zone to be well prepared for confident assessment of (Q)SAR and read-across predictions provided for the assessment of PPPs.

The workshop revolved around the evaluation of potential toxicological concern of groundwater metabolites and applicability of *in silico* methods as an extension of the WoE approach in the assessment of PPPs, e.g. in prediction of missing data on skin sensitisation of co-formulants. By increasing the understanding of *in silico* methods, building knowledge and make it more approachable, it was anticipated that the likelihood of data gaps being addressed through non-testing methods could be increased, reducing animal testing.

5.2. Objective

By having the chance to meet face-to-face, collaboration between the member states participating in the NZ work-sharing on assessment of PPPs is enforced. The objective of this workshop was for member states within the NZ to be more comfortable evaluating *in silico* predictions such as (Q)SAR and read-across when assessing relevance of groundwater metabolites, and also to facilitate taking such methods into consideration in an WoE approach for assessing acute toxicity of PPPs when applicable. In addition, the aim was to give an introduction to possible future application of *in silico* predictions. Thus, the intended outcome of the workshop was to:

- ✓ Provide an outline for applicability of (Q)SAR predictions/read-across and its limitations in a regulatory setting for PPPs.
- ✓ Assess examples of (Q)SAR and read-across data from past applications and applying the newly developed QSAR assessment framework.
- ✓ Assess situations (or future needs) where (Q)SAR and read-across data might be applied in relation to CLP calculations of acute toxicity or for grouping of PPP chemicals

5.3. Summary

OECD (Q)SAR Assessment Framework was first published in August 2023[5], including new OECD principles for evaluation of (Q)SAR predictions and for the final result for multiple predictions. The existing reporting formats, QMRF and QPRF, were also updated and QPRF in particular went through a major update.

In QAF, the OECD principles are broken down in assessment elements (AEs) and each AE is assessed by weighing importance, outcome and uncertainty. The overall uncertainty is based on AEs with the highest

importance. The risk assessor will decide the outcome of the prediction by deciding whether the prediction is acceptable for the intended regulatory purpose. By having a systematic description of the steps in QAF regulators, users and (Q)SAR model developers will all benefit. Regulators will indeed benefit from the transparency in the QMRF filled in by the model developers and the QPRF filled in by the (Q)SAR user, which again will result in better alignment in the assessments regulators in between.

The regulatory use of *in silico* predictions for PPPs is still limited. In a study by Benigni et al, 2020[6], it was found that (Q)SAR and read-across were only valid as stand-alone for prediction of Ames test for mutagenicity. However, (Q)SAR can be combined with other experimental data, such as NAMs, as lines of evidence in a WoE assessment. An example is the defined approach for skin sensitisation. Also, (Q)SARs for acute oral toxicity and skin sensitisation as stand-alone are evolving quickly, resulting in higher reliability. In other cases, e.g. assessments dealing with impurities or groundwater metabolites, *in silico* predictions may be the only option available to address the toxicity.

In silico tools may also contribute to a better mechanistic understanding of the molecular mode of action, and these methods are often cheaper and faster to apply compared to experimental data. However, from a regulatory view point, assessment of multiple predictions for many endpoints may be time consuming.

5.4. Conclusions

The workshop sparked some good discussions in the NZ toxicology group and the combination of introduction to the use of QAF and other relevant guidance documents, together with a case study was very successful. Within the NZ toxicology group it was discussed how the NZ guidance document[7] could better reflect the use of *in silico* methods and that the use of QMRF and QPRF reporting formats should be encouraged.

6. Specific recommendations from the workshop

- ✓ Regulatory risk assessors should encourage applicants to use the reporting formats QMRF and QPRF
- ✓ The QAF is a help for regulatory risk assessors also in terms of assessing the regulatory relevance of *in silico* predictions
- ✓ *In silico* methods as stand-alone is currently limited to prediction of the Ames test, but may be used for other endpoints as lines of evidence in a WoE assessment

7. Ongoing and follow-up in silico activities

Doris Hirmann mentioned that a new reporting format named QRRF, for reporting of the (Q)SAR result. The (Q)SAR result is the assessment of a property of a substance based on multiple (Q)SAR predictions. QRRF was included in the second version of QAF published November 2024[5].

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[6] Benigni, R., Serafimova, R., Morte, J.M.P., Battistelli, C.L., Bossa, C., Giuliani, A., Fioravanzo, E., Bassan, A., Gatnik, M. F., Rathman, J., Yang, C., Mostrag-Szlichtyng, A., Sacher, O. & Tcheremenskaia, O. (2020).Valuation of the applicability of existing (Q)SAR models for predicting the genotoxicity of pesticides and similarity analysis related with genotoxicity of pesticides for facilitating of grouping and read across: An EFSA funded project. *Regulatory Toxicology and Pharmacology*. Volume 114, 104658. https://doi.org/10.1016/j.yrtph.2020.104658.

[7] Northern Zone (Version 12, July 2024) Guidance document on work-sharing in the Northern zone in the authorisation of plant protection products. Retrieved from https://eng.mst.dk/media/wtbl5xnb/nzgd_2024_version_12_final_rev.pdf

EFSA is working on extending the genotoxicity database to include data on *in vitro* mammalian cell micronucleus test, and development of the OpenFoodTox 2.0 database includes integration of (Q)SAR platforms[8]. EFSA and ECHA are collaborating on a workflow to integrate the different available *in silico* tools in a meaningful way, including available (Q)SARs, the OECD (Q)SAR tool box, studies in IUCLID and metabolism studies that are in Metapath at the moment. They are also working on case studies, using actual dossiers, where member states can contribute. The aim is to finish this work in July 2026. A sub-group under the EFSA NAM working group is working specifically with (Q)SAR. The working groups OECD QSAR Expert Group and OECD MetaPath User Group are open to join, and the International Workshop on QSAR in Environmental and Health Sciences is also for regulatory toxicologists to participate in.

At the workshop a question was raised regarding the status of use of *in silico* tools for prediction of acute toxicity endpoints, especially dermal and inhalation toxicity. These endpoints are listed as data requirements under regulation (EU) No. 284/2013 for assessment of PPPs. This question was forwarded to the OECD (Q)SAR Expert Group. For acute oral toxicity there are available models performing well, however it is more difficult when it comes to mechanistic models of acute oral toxicity. A better mechanistic understanding helps in terms of regulatory acceptance of the models. Acute dermal and inhalation toxicity may even be more difficult to model than acute oral toxicity.

8. Biographies of the speakers

8.1. Doris Hirmann (ECHA)

Doris Hirmann is a senior expert in the Alternative Methods Team at the European Chemicals Agency (ECHA). With a background in chemistry, Doris specialised in the application of (Q)SAR models and profiling tools to predict chemical properties and environmental behaviour. Doris is dedicated to promoting the adequate use of QSAR. She contributed to the development of the OECD QSAR Toolbox, and currently works on implementing the QSAR Assessment Framework at ECHA. Additionally, she co-chairs the PBT Expert group and works on advancing approaches for assessing bioaccumulation.

8.2. Juan Manuel Parra Morte (EFSA)

Juan Parra, Scientific Officer at EFSA holding a MSc in Toxicology, Veterinary Medicine and Food Science and Technology with academic research in Genetic Toxicology. Expertise in Regulatory Toxicology in the EU peer review of pesticide active substances and their metabolites, with particular interest on *in silico* toxicology, and their implementation in the regulatory context. Juan has been involved in the development of guidance in the area of genotoxicity, endocrine disruption, isomers, water treatment transformation products and residue definition. Juan is active member of the OECD Expert Group on QSARs.

[8] Cattaneo, I., Astuto, M. C., Binaglia, M., Devos, Y., Dorne, J. L. C.M., Agudo, A. F., Dumont, A.F., Garcia-Vello, P., Kass, G. E.N., Lanzoni, A., Liem, A.K. D., Panzarea, M., Paraskevopulos, K., Morte, J.M.P., Tarazona, J.V. & Terron, A. (2023). Implementing New Approach Methodologies (NAMs) in food safety assessments: Strategic objectives and actions taken by the European Food Safety Authority. *Trends in Food Science & Technology*. Volume 133, 2023, Pages 277-290. https://doi.org/10.1016/j.tifs.2023.02.006.

8.3. Eva Bay Wedebye (DTU)

Eva Bay Wedebye, Senior Officer, Head of Research Group, National Food Institute Research Group for Chemical Risk Assessment and GMO, Technical University of Denmark (DTU), is an expert in the field of (Q)SARs. Eva has worked with development and regulatory application of Q)SARs for many different *in vivo* and *in vitro* endpoints since the end of the 1990s. Her team at the DTU Food Institute develops the freely available Danish (Q)SAR Database website (https://qsar.food.dtu.dk) with predictions from many (Q)SAR models for 650,000 substances and the >60 models available in the sister Models website for users to generate new predictions. She does research-based advise on (Q)SAR and other non-test methods for the Danish Environmental Protection Agency. This includes both assessments of specific substances as well as more generic advice such as commenting on EU and OECD guidance documents relating to use of non-testing methods. She is a member of the OECD QSAR Expert Group and the OECD (Q)SAR Assessment Framework Group, as well as EFSA groups on application of (Q)SARs for pesticide metabolites and botanical constituents.

9. Presentations

Doris Hirmann from ECHA, opened the workshop and introduced the key components in OECD (Q)SAR Assessment Framework. Doris placed the QAF in context of existing guidance and principles, and introduced the participants to five new principles in QAF related to evaluation of (Q)SAR predictions and results. While existing principles have focused on the validity of the model, QAF guides regulatory assessors to assess the reliability of the prediction and helps assessors to decide whether the outcome is fit for purpose for the specific regulation. In addition, QAF also covers the evaluation of multiple predictions, which initially are assessed as individual predictions, which finally are combined to ideally reach one correct determination of a final (Q)SAR result.

Doris introduced how the QAF is broken down in different AEs. Each assessment element is assessed by checking whether the outcome was fulfilled, how much weight the AE has in the assessment (low, medium, high), how much uncertainty is associated with the AE (low, medium, high) and a conclusion on whether the result is acceptable.

Doris also highlighted the importance of using the published and updated reporting formats QMRF and QPRF[9], which are interconnected with the steps in QAF. In addition, it is important to remember that when you have a checklist, it is very easy to find problems and issues. However, the goal is to increase the use NAM and ECHAs view is that NAMs should be used whenever it can. The QAF should not be used as a rejection checklist. Instead, QAF should be used to support consistency and quality.

After the introduction to QAF, Juan Manuel Parra Morte from EFSA presented EFSAs perspective on the use of *in silico* tools in the assessment of PPPs. Juan focused particularly on the use of (Q)SAR and read across in the assessment of pesticide groundwater metabolites and impurities in pesticide active substances. Juan gave an overview of available databases and guidance documents for assessing (Q)SAR and read across, and

[9] In addition to QMRF and QPRF, QRRF was included in the second version of QAF published November 2024 after the workshop

highlighted an EFSA supported project on the applicability of (Q)SAR and read-across for genotoxicity assessment of pesticide residues by Benigni et al.[6] The conclusion from the project was that only (Q)SAR prediction of Ames test for mutagenicity could be used as stand-alone. Juan mentioned further that (Q)SAR and read across may be used as lines of evidence together with additional information for prediction of other endpoints, and that (Q)SARs as stand-alone for assessment of oral toxicity[10] and skin sensitisation are evolving quickly.

Juan gave an update on ongoing activities: extension of the EFSA Pesticide Genotoxicity Database[11,12], and collaboration between EFSA and ECHA on a workflow to integrate the different tool in a meaningful way (including (Q)SARs, the OECD tool box, studies in IUCLID and metabolism studies in MetaPath and a subgroup on (Q)SAR under EFSA working groups on NAM).

On the second day, Jonas Tägt from the Swedish Chemicals Agency (KEMI) presented a case example applying the QAF to assess a (Q)SAR prediction of acute toxicity of groundwater metabolites. Through the case study, the participants got hands-on experience going through the AEs in QAF. Based on the case example, a fruitful discussion was started. Among the topics was the selection of endpoints to predict the underlying mechanism.

In the afternoon, a Q&A session had been organised with participation from Eva Bay Wedebye (DTU). Eva explained how (Q)SAR is validated and related pitfalls, such as the size of external validation datasets. She also mentioned how the (Q)SAR models can tolerate a certain degree of false errors in the dataset until it collapses, as long as the model can find the trends in the data. Eva pointed out that improvement within the field of *in silico* tools is especially related to transparency and data treatment before the modelling and on what the model can predict. She also pointed out that the QAF, together with the reporting formats QMRF and QPRF, offers much help to the regulators. In addition, the QAF can also be viewed as a tool for transparency in the assessment. Eva further mentioned ongoing work on development on models for prediction of skin sensitisation.

10. Acknowledgements

We would like to thank all speakers for sharing their invaluable knowledge, and all NZ tox participants for active discussions. We would also like to thank NKE for funding the workshop and providing the opportunity for meeting and discussing points of interest within the Northern Zone toxicology group. Finally, a special thanks to Karina Arnhild for support in organising the workshop.

[10] Bishop, P.L., Mansouri, K., Eckel, W.P., Lowit, M.B., Allen, D., Blankinship, A., Lowit, A.B., Harwood, D.E., Johnson, T. & Kleinstreuer, N.C. (2024) Evaluation of in silico model predictions for mammalian acute oral toxicity and regulatory application in pesticide hazard and risk assessment. *Regulatory Toxicology and Pharmacology*, Volume 149, 105614. https://doi.org/10.1016/j.yrtph.2024.105614.

[11] Metruccio, F., Castelli, I., Civitella, C., Galbusera, C., Galimberti, F., Tosti, L. & Moretto, A. (2017). Compilation of a database, specific for the pesticide active substance and their metabolites, comprising the main genotoxicity endpoints. *EFSA supporting publication*. 2017:EN-1229. 125 pp. doi:10.2903/sp.efsa.2017.EN-1229

[12] Metruccio, F., Castelli, I., Civitella, C., Galbusera, C., Galimberti, F., Tosti, L. & Moretto, A. (2017, July 11th). *Database specific for the pesticide active substance and their metabolites, comprising the main genotoxicity endpoints*. Version V1. Retrived from https://zenodo.org/records/582137